J Mol Med (1999) 77:640–655
Digital Object Identifier (DOI) 10.1007/s001099900042

REVIEW

Rong-Fu Wang

Human tumor antigens: implications for cancer vaccine development

Received: 6 April 1999 / Accepted: 10 August 1999 / Published online: 8 September 1999

Abstract The adoptive transfer of tumor-infiltrating lymphocytes along with interleukin 2 into autologous patients resulted in the objective regression of tumor in about 30% of patients with melanoma, indicating that these T cells play a role in tumor rejection. To understand the molecular basis of the T cell-cancer cell interaction we and others started to search for tumor antigens expressed on cancer cells recognized by T cells. This led to the identification of several major histocompatibility complex (MHC) class I restricted tumor antigens. These tumor antigens have been classified into several categories: tissue-specific differentiation antigens, tumor-specific shared antigens, and tumor-specific unique antigens. Because $CD4^+$ T cells play a central role in orchestrating the host immune response against cancer, infectious diseases, and autoimmune diseases, a novel genetic approach has recently been developed to identify these MHC class II restricted tumor antigens. The identification of both MHC class I and II restricted tumor antigens provides new opportunities for the development of therapeutic strategies against cancer. This review summarizes the current status of tumor antigens and their potential applications to cancer treatment.

Key words Melanoma · T cell recognition · Major histocompatibility complex · Immunotherapy · Epitopes

Abbreviations *APC* Antigen-presenting cells · *CDK* Cyclin-dependent kinase · *CTL* Cytotoxic T lymphocytes · *DC* Dendritic cells · *EBV* Epstein-Barr virus · *GnT-V N*-Acetylglucosaminyltransferase V · *IL-2* Interleukin 2 · *LDFP* Low-density-lipid receptor fusion protein · *MHC* Major histocompatibility complex · *ORF* Open reading frame · *PBMC* Peripheral blood mononuclear cells · *SCP* Synaptonemal complex protein · *TIL* Tumor-infiltrating lymphocytes · *TPI* Triosephosphate isomerase · *TRP* Tyrosinase-related protein

RONG-FU WANG received his Ph.D. in molecular genetics at the University of Georgia and completed his postdoctoral fellowship in the Department of Microbiology and Immunology at Stanford University School of Medicine. Since 1996 he has been a principal Investigator in the Surgery Branch, National Cancer Institute. His research interests include tumor antigens and cancer vaccine development, cDBA microarray, and gene transfer technology.

R.-F. Wang
National Cancer Institute, NIH, Building 10/2B42,
Bethesda, MD 20892, USA
e-mail: rongfu@pop.nci.nih.gov
Tel.: +1-301-4961437, Fax: +1-301-4960011

Introduction

The immune system plays a critical role in immune surveillance against cancer and other diseases. The importance of T cells in antitumor immunity has been demonstrated in experimental animal tumor models [1, 2, 3]. Adoptive transfer of tumor-infiltrating lymphocytes (TIL) along with interleukin 2 (IL-2) into the autologous patients also has demonstrated an important role of T cells in tumor regression [4, 5]. To understand how T cells mediate antitumor responses, and what molecules on tumor cells are recognized by T cells, a great deal of effort has been made to identify tumor antigens recognized by cytotoxic T lymphocytes (CTL) with antitumor reactivity in vivo and in vitro. The identification of a number of MHC class I restricted tumor antigens derived from melanoma and other types of cancer has provided the basis for understanding T cell mediated antitumor activity and a rationale for the development of anticancer vaccines.

Because tumor specific $CD8^+$ CTL lyse tumor cells directly and can eradicate large tumor masses in vivo,

the approach used for immunotherapy of cancer is limited to the role of $CD8^+$ CTL. Moreover, it has been difficult to identify MHC class II restricted antigens due to differences in the antigen processing and presentation of MHC class II restricted antigens from the MHC class I pathway. As a result little attention has been paid to $CD4^+$ T helper cells, and only few MHC class II restricted tumor antigens have been identified thus far. However, the important role of CD4 T cells in priming and maintaining of antitumor immune responses has been documented in several animal tumor models [6, 7, 8, 9]. $CD4^+$ T cells also play a role in controlling tumor growth and protecting mice from tumor challenge in MHC class II negative tumor model [10]. Vaccination of mice with a tumor specific CTL epitope and a $CD4^+$ T cell epitope produced a potent antitumor immunity. These studies suggest the importance of both $CD8^+$ and $CD4^+$ T cells in antitumor immune responses. Therefore the identification of MHC class II restricted tumor antigens is critical for the development of more effective cancer vaccines.

Identification of tumor antigens has led to several clinical trials. Peptide-based vaccines and recombinant viruses encoding tumor antigens have been evaluated in the treatment of patients with melanoma [11, 12]. This review summarizes the current status of human tumor antigens and discusses the clinical applications of these tumor antigens in cancer therapy.

Methods for the identification of MHC class I restricted tumor antigens

cDNA expression cloning

The cDNA expression system is to transfect cDNA libraries into cells expressing the appropriate MHC class I molecule and to screen positive pools with tumor reactive T cells based on either cell lysis or cytokine release [13]. This approach has been improved by using cDNA pools along with a plasmid encoding an appropriate MHC class I into COS-7 or 293 cells [14, 15, 16]. Major limitations of this approach include the requirement to determine the MHC restriction element prior to library screenings and the need to identify the antigenic peptides based on the amino acid sequence predicted from the open reading frame (ORF) of a gene.

Retrovirus-based cDNA expression system

To overcome the problems associated with difficulties in identifying the MHC restriction element utilized by $CD8^+$ CTL because the blocking antibodies specific for particular MHC alleles are unavailable, or the tumor antigen recognized by the CTL is a unique or mutated antigen, we recently developed a retrovirus-based cloning system. The major obstacle for cloning antigens recognized by T cells with an unknown restriction element is the lack of efficient introduction of cDNA library pools into autologous fibroblast or Epstein-Barr virus (EBV) B cells, and the traditional cDNA expression systems cannot be applied to these cells. The retroviral-based cDNA expression system allows us efficiently to introduce a cDNA library into autologous fibroblasts which are then capable of processing and presenting antigenic peptides to T cells [17]. The screening strategy is shown in Fig. 1. First, a retroviral cDNA library is constructed, and divided into cDNA pools consisting of approximately 30 colonies in a 96-well plate format. Second, we transiently transfect retroviral cDNA library pools plus a plasmid encoding vesicular stomatic virus G protein into 293GP packaging cell lines. Two days after transfection virus supernatants are harvested and used to infect cultured autologous fibroblasts. After 2 days incubation T cells are added to the transduced fibroblasts, and positive retroviral cDNA pools are identified by measuring cytokine release from T cells. The use of relatively small cDNA pools obviates the need to generate a retrovirally transduced stable cell library or cell clones and the need to isolate cDNA inserts encoding tumor antigens from genomic DNA. The usefulness of this system is currently being tested to identify new antigens recognized by $CD8^+$ T cells without knowledge of restriction element.

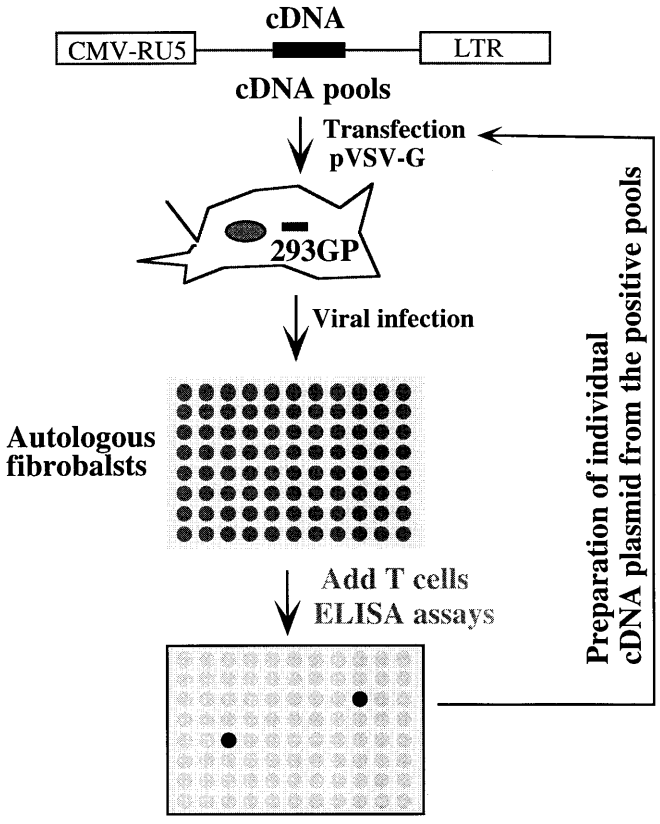

Fig. 1 Schema for expression cloning using retrovirus-based cDNA libraries. *pVSV-G* Plasmid-encoding vesicular stomatic virus G protein; *LTR* long terminal repeat; *CMV* cytomegalovirus

Peptide elution and combinatorial peptide library

Tumor-specific peptides can be eluted with acid from either the tumor cell surface or purified peptide-MHC complexes, and subsequently separated by high-pressure liquid chromatography. The eluted peptide fractions are then tested for their ability to stimulate cytokine secretion from CTL when pulsed onto the MHC matched antigen-presenting cells (APC). A naturally processed tumor-specific peptide recognized by CTL can be directly identified [18] after sequence determination by Edman degradation or tandem mass spectrometry. The peptide sequence can then be used to search databases for find the gene encoding the antigenic peptide. Drawbacks to this approach include the technical complexity associated with fractionation of the active peptides and the use of instruments such as tandem mass spectrometry.

An alternative approach is to use synthetic peptide libraries based on the peptide-binding motif of a particular MHC molecule. This has been used for identification of an antigenic peptide recognized by a CTL line [19]. However, the identified peptide may be a cross-reactive peptide rather than an authentic peptide derived from a tumor antigen.

"Reverse immunology" approach

Few tumor antigens have been isolated from breast, prostate, and other types of cancer due to the lack of tumor reactive T cells, but there are putative tumor antigens whose expression is known to be associated with tumor [20, 21]. Recently, serological analysis of recombinant cDNA expression library of human tumor with autologous serum (SEREX) has been used to isolate many putative human tumor antigens [22, 23]. Among these are tyrosinase [24], MAGE [24], NY-ESO-1 [25], SSX2 [26], synaptonemal complex protein (SCP-1) [27], and CT7 [28]. Interestingly, tyrosinase, MAGE, and NY-ESO-1 are the T cell defined antigens [14, 29, 30, 31], suggesting that SEREX analysis is useful in identifying tumor antigens which may be recognized by antibodies and T cells. HOM-TES-14 was identified by screening a testis cDNA library with high-titered IgG antibodies from the serum of a patient with renal cell carcinoma and proved identical to SCP-1 [27]. SCP-1 is known to be expressed selectively during the meiotic prophase of spermatocytes and is involved in the pairing of homologous chromosomes. It is frequently expressed in malignant gliomas, breast, renal cell, and ovarian cancer [27]. CT7 is a newly defined gene with partial sequence homology to the MAGE family at its C-terminus and contains a distinct repetitive sequence at the 5' end [28]. T cell reactivity for SCP-1 and CT7 has not been demonstrated. However, since the production of antibodies against theses antigens requires both B and $CD4^+$ T cells, it is possible that T cell epitopes exists in these antigens.

To validate an antigen identified by SEREX or by other approaches based on specific expression pattern as a true tumor antigen, it is necessary to generate $CD4^+$ T cells and CTL in vitro using peptides derived from a putative tumor antigen [21, 32, 33, 34]. Although many strategies have been developed using peripheral blood mononuclear cells (PBMC) or dendritic cells (DC) pulsed with peptides or purified proteins as stimulators, it is not clear which method is better for efficient generation of CTL in vitro. In many cases these CTL recognize the peptide used for stimulation but do not recognize the tumor cells [35]. Several factors could account for the inability of these T cells to recognize tumor cells: (a) the affinity of peptide-reactive CTL may be too low to recognize the small amounts of peptide naturally displayed at the cell surface; (b) the peptide used in vitro systems may not be naturally processed and presented on the tumor cell surface. Recent studies show that MAGE 3-specific CTL recognize melanoma pulsed with MAGE-$3_{271–279}$ peptide and cells transfected with a minigene encoding the preprocessed fragment MAGE-$3_{271–279}$ but fail to recognize melanoma expressing the full-length MAGE-3 protein [36]. Pretreatment of melanoma cells expressing MAGE-3 with protease inhibitors results in efficient lysis by MAGE-$3_{271–279}$ specific CTL [36]. Another study found that mutational hotspots in p53 alter proteasomal processing and block to generate a proper T cell epitope [37]. These studies suggest that regulation of proteasomal processing is important for generating proper or new T cell epitopes on tumor cells recognized by CTL. Although significant progress has been made in understanding the rules of peptide-binding motifs for various MHC molecules [38], the predicted peptides which contain the MHC-binding motif may not, however, be naturally processed and presented on tumor cells at sufficient levels to be recognized by T cells. To avoid these problems, the use of APC such as DC pulsed with purified whole protein or transfected with a cDNA encoding the putative tumor antigen as stimulators should be subjected to further investigation. Alternatively, HLA-A2 transgenic mice could be used as a natural selection system to generate tumor reactive T cells. Once the tumor antigen reactive T cells are produced, T cell epitopes can be defined by testing T cell recognition for synthetic peptides. The HLA-A2 transgenic mice have been successfully used to generate HLA-A2 restricted CTL [39, 40].

Tumor antigens recognized by MHC class I restricted $CD8^+$ T cells

Tissue-specific shared differentiation antigens

Tyrosinase is the first member of differentiation antigens identified (Table 1) and is an important enzyme involved in the synthesis of melanin (Fig. 2) [14, 41]. Several distinct HLA-A2 restricted T cell epitopes have been identified from the tyrosinase molecule [42, 43]. Tyrosinase has also been shown to be reactive with TIL 888, which resulted in the regression of multiple metastatic lesions

Table 1 Tissue-specific differentiation antigens

Antigens	HLA restrictions	Peptides[a]	References
Tyrosinase	HLA-A2	MLLAVLYCL	14, 42
	HLA-A2	YMNGTMSQV	43
	HLA-B44	SEIWRDIDF	46
	HLA-A24	AFLPWHRLF	45
	HLA-A1	KCDICTDEY	59
	HLA-A1	SSDYVIPIGTY	59
MART-1/Melan-A	HLA-A2	AAGIGILTV	49
	HLA-A2	EAAGIGILTV	49
	HLA-B45	AEEAAGIGILTV	52
gp100	HLA-A2	KTWGQYWQV	54
	HLA-A2	ITDQVPFSV	54
	HLA-A2	YLEPGPVTA	54
	HLA-A2	LLDGTATLRL	54
	HLA-A2	VLYRYGSFSV	54
	HLA-A2	RLMKQDFSV	54
	HLA-A2	RLPRIFCSC	54
	HLA-A3	SLIYRRRLMK	59
	HLA-A3	ALLAVGATK	59
	HLA-A2	VYFFLPDHL (intron 4)	122
gp75/TRP-1	HLA-A31	MSLQRQFLR (ORF3)	16
TRP-2	HLA-A31	LLPGGRPYR	65
	HLA-A33	LLPGGRPYR	70
	HLA-A2	SVYDFFVWL	71
	HLA-A68	EVISCKLIKR (intron 2)	123

[a] Single amino acid codes were used for peptide sequences

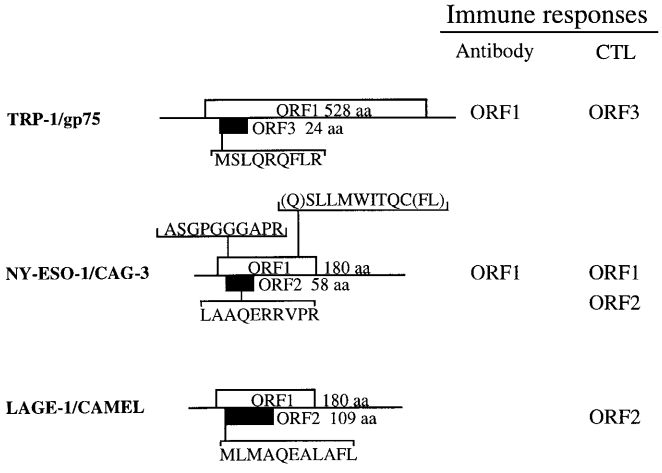

Fig. 2 The use of alternative ORFs for generating T cell epitopes. The primary ORFs are used to transalate the primary gene products (*ORF1*). Alternative ORFs are located within the coding region of *ORF1*. Antibody response from sera of patients with melanoma have been detected to recognize gp75 or NY-ESO-1. T cell epitopes derived from either ORF1, ORF2, or ORF3 are indicated in amino acid sequences

when adoptively transferred into the autologous patient along with IL-2 [44, 45]. In addition, the peptides derived from the tyrosinase protein were recognized by HLA-B44 and HLA-A1 restricted T cells [46]. Interestingly, a naturally processed HLA-A2 associated peptide, YMDGTMSQV, was identified by peptide elution and mass spectrometry. This peptide results from the posttranslational conversion of asparagine to aspartic acid [47].

The MART-1 gene was isolated by screening of a melanoma cDNA library with the HLA-A2 restricted melanoma-reactive TIL 1235 [48]. MART-1 is identical to Melan-A, independently cloned by another group [15]. The MART-1 cDNA isolated from melanoma cells contain no mutation or other alterations in the coding region. Northern blot analysis indicated that MART-1 was expressed in melanomas, melanocytes, and retina but not in normal human tissues [48]. The MART-1 gene product was found to be an immunodominant antigen recognized by the majority of HLA-A2 restricted melanoma reactive CTL established this laboratory [48, 49] as well as a large percentage of melanoma-reactive clones derived from the peripheral blood lymphocytes of HLA-A2$^+$ melanoma patients [50]. Both 9-mer peptide, AAGIGILTV, and 10-mer peptide, EAAGIGILTV, were found to be recognized by HLA-A2 restricted melanoma-specific CTL [49, 51]. A tumor-reactive T cell line was recently reported to recognize MART-1 peptide in the context of HLA-B45 [52]. Although MART-1 is a dominant antigen recognized by many HLA-A2 restricted TIL, no correlation has been found between T cell recognition of MART-1 and clinical response. One study found that the MART-1 reactive CTL recognized the MART-1 mimic peptides derived from a number of proteins including bacterial and viral proteins [53]. This provides a possible explanation of why a high precursor frequency of MART-1 reactive T cells is present in PBMC of normal and patients with melanoma. In contrast, a strong correlation was found between the T cell recognition of gp100 and clinical response [54].

A gene encoding gp100 was isolated by the use of tumor-reactive CTL as well as a tumor-reactive antibody [55, 56, 57]. Northern blot analysis showed that gp100 is expressed in neonatal cultured melanocyte lines, most melanoma cell lines, and retina but not in other normal

tissues [57]. The gp100 molecule was found to be recognized by 8 of 21 HLA-A2 restricted melanoma reactive TIL established from various patients with melanoma in the Surgery Branch, NCI, and appears to represent a highly immunogenic antigen. Several T cell epitopes have been defined by the use of CTL derived from various patients [54, 57, 58, 59]. Recently, two additional HLA-A3 restricted epitopes, ALLAVGATK and LIYRRRLMK, were identified from the normal coding sequence of gp100 [59, 60].

One of the gp100 epitopes, YLEPGPVTA, was independently isolated from an high-pressure liquid chromatography purified fraction of peptides obtained from HLA-A2$^+$ melanoma [18]. This observation validates both genetic and biochemical approaches to the identification of naturally processed peptides on tumor cells.

Adoptive transfer of TIL 586 with IL-2 into the autologous patient resulted in an objective regression of tumor. A tumor antigen encoded by tyrosinase-related protein (TRP)-1/gp75 was isolated following cDNA library screening using TIL 586 [16]. Interestingly, gp75 was previously reported to be recognized by IgG antibodies in the serum of a patient with melanoma [61]. Northern blot and protein analysis indicated that the gp75 protein is one of the most abundant intracellular glycoproteins in melanocyte-lineage cells but was not detected in non-melanocytic cell types [62, 63]. The gp75 molecule has recently been shown to have DHI-2 carboxylic acid oxidase activity involved in the synthesis of melanin [64]. Interestingly, the T cell epitope was identified from a gene product translated from alternative ORF 3 (see below).

To test whether TRP-1/gp75 is the only antigen recognized by TIL 586, a number of CTL clones were established from TIL 586. The recognition pattern of CTL clones from TIL 586 could be classified into several categories: the first class of CTL clones recognized TRP-1/gp75, 586mel, and HLA-A31$^+$ melanocytes; the second class of CTL clones did not recognize TRP-1/gp75, but strongly recognized autologous tumor and HLA-A31$^+$ normal melanocytes, suggesting that these CTL clones recognized a new antigen; the third class of CTL clones recognized autologous tumor, but neither TRP-1/gp75 nor HLA-A31$^+$ normal melanocytes. This led to identification of TRP-2 as a tumor antigen recognized by CTL clones from TIL 586 (Table 1) [65]. TRP-2 is a member of the tyrosinase related protein family and has approximately 40–45% of amino acid sequence identity to tyrosinase or TRP-1/gp75. An antigenic peptide was then identified from the normal ORF of TRP-2. Interestingly, a mouse TRP-2 was identified as a tumor antigen recognized by CTL reactive with B16 melanoma [66, 67]. This represents the first example that both human and mouse TRP-2 act as tumor antigens. Therefore the mouse TRP-2 can be tested as an ideal antigen for studies important for human cancer vaccines.

To increase the utility of tumor antigens such as TRP-1 and TRP-2 for immunotherapy it is important to identify T cell epitopes restricted by other MHC class I alleles. Based on the structural similarities of a group of HLA alleles and peptide-binding motifs, several supertypes have been proposed [68, 69]. The HLA-A31 molecule belongs to a member of the HLA-A3-like supertype, which covers 45–50% of all ethnic populations. A recent study demonstrated that both TRP-1 and TRP-2 peptides are capable of binding to HLA-A3, HLA-A11, HLA-A31, HLA-A33 and HLA-A68 [70]. Furthermore, the TRP-2 peptide was recognized by T cells in the context of HLA-A31 and HLA-A33 [70]. These studies raised a possibility that the TRP-1 and TRP-2 peptides could be used in the peptide-based vaccines to treat patients expressing one of the HLA-A3 superfamily members. In addition, an HLA-A2 restricted CTL line was generated from PBMC by multiple stimulation in vitro with synthetic peptides. These T cells recognized HLA-A2$^+$/TRP-2$^+$ tumor lines as well as T2 cells pulsed with a TRP-180–188 peptide [71]. Because this peptide is identical to one identified from mTRP-2, it is useful to test it for the development of cancer vaccines in a B16 tumor model.

Tumor-specific shared antigens

MAGE-1 is the first tumor antigen identified on a human melanoma using a genetic approach [29]. MAGE-1 was isolated from the transfectants on the basis of its ability to stimulate cytokine release from the CTL [29]. The peptide epitope, EADPTGHSY, was subsequently identified and recognized by CTL in the context of HLA-A1 (Table 2) [72]. A second antigenic peptide was identified from the MAGE-1 protein and recognized by HLA-Cw16 restricted CTL [73]. The MAGE-1 gene is expressed in approximately 30% of melanomas as well as in other types of tumors, but is not detected in the normal human tissues with the exception of testis [29].

DNA hybridization analysis indicated that MAGE-1 is a member of a multiple-gene family. A similar peptide, EVDPIGHLY, derived from MAGE-3, was shown to be recognized by HLA-A1 restricted CTL [74]. Additional peptides were identified with HLA-A2 and B44 restricted CTL generated in vitro with a synthetic peptide derived from MAGE-3 [75, 76]. MAGE-3 is not expressed in human normal tissues except testis. Because MAGE-3 expression is detected in approximately 60% of melanomas, it may represent a good candidate for the development of vaccine strategies.

Two additional antigens were identified from a cDNA library made from the same MZ2 melanoma cell line (Table 2). A cDNA clone encoding BAGE was isolated using a class I HLA-Cw16 restricted T cell clone derived from the MZ2 patient [77]. This gene encodes a polypeptide of 43 amino acids and appears to be expressed in a similar pattern to members of the MAGE family. A peptide of nine amino acids was recognized by CTL in the context of HLA-Cw16. Using the same approach, a gene encoding an antigen recognized by a class I HLA-Cw6 restricted T cell clone was isolated. This led to the iden-

Table 2 Tumor-specific shared antigens

Antigens	HLA restrictions	Peptides	References
MAGE-1	A1	EADPTGHSY	72
	Cw16	SAYGEPRKL	73
MAGE-3	A1	EVDPIGHLY	74
	A2	FLWGPRALV	75
	B44	MEVDPIGHLY	76
GAGE	Cw6	YRPRPRRY	78
	A29	YYWPRPRRY	82
BAGE	Cw16	AARAVFLAL	77
RAGE	B7	SPSSNRIRNT	85
NY-ESO-1/CAG3			
ORF1	A2	(Q)SLLMWITQC(FL)	30
	A31	ASGPGGGAPR	31
ORF2	A31	LAAQERRVPR	31
LAGE-1/CAMEL			
ORF2	A2	MLMAQEALAFL	118

Table 3 Unique/mutated tumor antigens

Antigens	HLA restrictions	Peptides	References
CDK4	A2	ACDPHSGHFV	91
MUM-1	B44	EEKLIVVLF	90
β-Catenin	A24	SYLDSGIHF	93
Caspase-8	B35	FPSDSWCYF	101

tification of a new family of genes named GAGE [78]. Several GAGE-related genes have been reported [79, 80, 81, 82]. Two T cell epitopes from GAGE genes were identified and recognized by CTL in association with HLA-Cw*0601 [78] as well as HLA-A29 [82]. GAGE genes were found to have an expression pattern similar to that of the MAGE and BAGE genes [78].

NY-ESO-1/CAG-3 is another antigen in this group. Several CTL clones established from TIL 586 did not recognize TRP-1 or TRP-2 or normal HLA-A31$^+$ melanocytes, but they still strongly recognized the autologous tumor 586mel [65]. After screening a cDNA library, several positive cDNA clones were identified [31]. DNA sequence analysis revealed that this gene, designated as CAG-3, is identical to NY-ESO-1, which was first reported to be recognized by the autologous serum from a patient with esophageal cancer [25]. NY-ESO-1 has also been reported to be recognized by CTL generated from blood lymphocytes in vitro in an HLA-A2 restricted fashion [30]. Both HLA-A2 and HLA-A31 restricted T cell epitopes have been identified from its primary ORF (Table 2) [30, 31]. This gene is not expressed in normal human tissues except testis but is frequently expressed in melanoma (30%), breast (25%), prostate (25%), bladder (80%), lung carcinoma (50–70%), and other types of cancers [25, 65, 83]. Serological analysis indicated that approximately 10% of patients with cancer developed antibodies against NY-ESO-1 protein, suggesting that this is an immunogenic antigen [84]. HLA-A31 restricted CTL has been shown to recognize NY-ESO-1$^+$ melanoma and breast and lung cancers [31, 83].

RAGE was recently identified with CTL reactive to a renal cell carcinoma [85]. This gene is expressed in a number of tumors of various histological types and normal retina but not other normal tissues.

Tumor-specific unique antigens

Many genetic mutations or alterations have been identified in cancer cells. These mutated proteins or peptides resulting from genetic mutations could be more immunogenic; it has been long assumed that many tumor antigens would be mutated antigens recognized by T cells. However, the majority of tumor antigens identified to date are nonmutated self-proteins. To test whether mutated ras and p53 are tumor-specific antigens, several groups have raised CTL against normal or mutated peptides from the *ras* proto-oncogene (67) and *p53* tumor suppressor gene [20, 39, 86, 87, 88, 89]. Nevertheless, several mutated gene products have been recently identified as tumor-specific antigens by screening cDNA libraries using tumor-reactive CTL (Table 3).

MUM-1 (melanoma-ubiquitous mutated) was isolated following screening cDNAs library derived from the LB33 melanoma cell line with an HLA-B44 restricted CTL [90]. DNA sequence analysis identified a point mutation in a cDNA which led to a change in one amino acid (Ser/Ile) at position 5 of the peptide. Since both the normal and mutated peptides bound efficiently to the class I HLA-B44 molecule, but only the mutated form could be recognized by T cells, the mutation appeared to be involved in T cell recognition.

A second mutated gene is cyclin-dependent kinase 4 (CDK4), an enzyme involved in cell cycle control. DNA sequence analysis indicated that a point mutation (a C/T transition) results in a substitution of a cysteine for an arginine residue at codon 24, generating a new epitope recognized by CTL [91]. The CDK4 protein usually forms a complex with cyclin D_1 and phosphorylates the pRB protein, and therefore promotes the cell cycle progression from G_1 to S phase [92]. However, assembly of CDK4 with cyclin D_1 as well as its kinase activity was found to be inhibited by $p16^{INK4a}$. Interestingly, $p16^{INK4a}$ cannot bind to the mutated CDK4 and fails to inhibit the kinase activity of CDK4/cyclin D [91], implying that the mutation in the CDK4 gene leads to a loss of cell cycle control. This suggests that a mutated tumor antigen also plays a role in tumorigenesis.

β-Catenin was isolated as a mutated antigen with TIL 1290 derived from a melanoma patient 888 [93]. Partial cDNA sequence analysis identified a point mutation which was responsible for a change of serine to phenylalanine in the coding region and constituted a new T cell epitope for T cell recognition. The β-catenin protein has been shown to be a cytoplasmic protein that interacts with the cellular adhesion molecule E-cadherin [94]. A number of mutations have been found in the β-catenin gene product from various tumors [95, 96]. Loss of cell adhesion molecules may play a role in the metastatic process [97]. Recently three groups reported that the upregulation or stabilization of β-catenin may contribute to tumorigenesis and cancer progression due to mutations in the adenomatous polyposis coli tumor suppressor protein (APC) or β-catenin [98, 99, 100]. More importantly, the point mutation initially identified by CTL screening of a melanoma cDNA library is identical to that in β-catenin identified in colon cancer [98, 99].

The mutated CASP-8 was recently identified with CTL specific for human squamous tumor [101]. The antigen encoded by caspase-8 is required for the induction of apoptosis through Fas-FasL and the tumor-necrosis factor pathway. A T cell epitope was identified from an extended C-terminus portion of protein resulting from the nucleotide substitution in the stop codon by the point mutation.

These results suggest that some mutated genes involved in cell cycle regulation and apoptosis may have biological functions in tumorigenesis in addition to serving as immune targets for T cell recognition. The identification of these mutated antigens is important for our understanding of molecular basis of cancer and for designing a specific strategy for cancer therapy.

Tumor antigens ubiquitously expressed

MUC-1 has been shown to be expressed on breast and pancreatic adenocarcinomas [102, 103] in addition to epithelial cells, fibroblasts, and B cells. MUC-1 has an unusual structure consisting of many copies of a 20 amino acid sequence repeat [104]. Because of increased levels of expression and decreased glycosylation, MUC-1 could provide a suitable target for immunotherapy of cancer. However, T cell recognition of the MUC-1 gene product appeared to be non-MHC-restricted and specific for tumor cells due to differences in protein structure and posttranslational modifications between tumor and normal cells [104]. The epitopes for T cell recognition were found in the tandem repeat of the MUC-1 protein [104]. Recently, MHC-restricted MUC-1 specific CTL have been generated and shown to recognize breast tumor. One peptide derived from the tandem repeat was found to be recognized by HLA-A2 restricted CTL [105], and the other recognized by HLA-A11, HLF-A1, and HLF-A3 restricted CTL [106]. Peptide mimics of a MUC-1 peptide have been shown to be effective in generating functional CTL [107]; therefore the modified peptides may be useful in breaking T cell tolerance and could provide a new approach for cancer vaccine development.

HER-2/neu is another shared tumor antigen recognized by T cells in breast and ovarian cancers. The *HER-2/neu* proto-oncogene encodes a tyrosine kinase protein whose expression has been shown to increase in 30% of breast and ovarian cancers. In breast cancer HER-2/neu overexpression is reported to be associated with aggressive disease. CTL isolated from tumor-associated lymphocytes can specifically recognize a synthetic peptide corresponding to amino acids 971–980 of the HER-2/neu protein [108]. This is the first demonstration that CTL isolated from human tumors recognize HER-2/neu as an ovarian tumor antigen. Recently four ovarian tumor reactive CTL were established from different $HLA\text{-}A2^+$ patients and were capable of recognizing both freshly isolated $HER\text{-}2/neu^+$ tumor cells and non-HLA-A2 ovarian tumor lines transfected with HLA-A2 cDNA [21]. A common epitope, KIFGSLAFL, was found to be recognized by four of four CTL lines [21]. Another epitope peptide (971–980) was also found to be recognized by two of four CTL lines. These results were supported by findings of other groups [109, 110]. Recognition and lysis of ovarian cancer cells by CTL were also shown to be correlates with the expression level of HER-2/neu in the tumor cells [109]. Most importantly, the breast and ovarian cancer specific CTL recognized the same epitope peptide (GP2; amino acids 654–662) derived from the HER-2/neu protein in the context of HLA-A2 [111, 112]. It appears that the GP2 peptide represents a common epitope shared by various epithelial tumors because it was recognized by CTL lines derived from breast, ovarian, non-small-cell lung, and pancreatic cancers [112]. Recently, a HLA-A3-restricted T cell epitope was identified from HER-2/neu by CTL-generated in vitro immunization with peptide-pulsed DC [113].

A new mechanism for generating T cell epitopes

A general rule is that one gene encodes one protein or one enzyme. However, in the course of characterizing T

cell epitopes encoded by TRP-1/gp75 we have realized that in some cases one mRNA is translated into two gene products by the use of alternative ORFs. A small DNA fragment encoding an antigenic peptide was initially identified by the deletion mapping, but the antigenic peptide recognized by TIL 586 could not be found from peptides derived from the primary reading frame of gp75 [114]. This led us to test the hypothesis that T cells recognize an antigenic peptide derived from the gene products translated from an alternative ORF of gp75. To our surprise, one peptide MSLQRQFLR which was derived from ORF3 of TRP-1/gp75 was capable of stimulating cytokine release from TIL 586 when pulsed onto HLA-A31$^+$ EBV-transformed B cells [114]. This represents the first demonstration of two overlapping ORFs being used to translate two distinct polypeptides from a single eukaryotic cellular mRNA for immunological recognition. Although the use of overlapping reading frames has been reported, these studies were limited to viral mRNAs using ORF-specific antibodies [115, 116]. Change of the start codon AUG to AUC of the alternative ORF3 resulted in the loss of T cell recognition, suggesting that the translation of ORF3 is essential for generating an epitope recognized by TIL 586. The translation of ORF3 of gp75 appears to be active in both tumor and normal melanocytes based on T cell recognition of these cells [114]. Thus, these results demonstrated a novel mechanism by which a human tumor antigen can be generated from an alternative ORF and presented to T cells by a MHC class I molecule.

The use of alternative ORFs was also found in NY-ESO-1. Several CTL clones established from TIL 586 recognized antigenic peptides derived from the primary ORF of NY-ESO-1, which encodes a protein of 180 amino acids. However, at least two other CTL clones derived from the same TIL 586 did not recognize peptides from the NY-ESO-1 protein but were still capable of recognizing COS-7 transfected with the NY-ESO-1 cDNA [31]. Further analysis demonstrated that NY-ESO-1 produces two gene products: 180 amino acid protein translated from the primary ORF and 58 amino acid polypeptide translated from ORF2. Therefore, two CTL epitopes have been identified from the gene product (180 amino acids), and one epitope from the second gene products (58 amino acids) encoded by ORF2 [31]. Furthermore, ORF2 specific CTL were capable of recognizing NY-ESO-1$^+$ melanoma as well as breast tumor [31].

More recently, LAGE-1 was originally identified by representational difference analysis [117] and shown to share 94% nucleotide and 87% amino acid homology to NY-ESO-1. A short form of LAGE-1, designated as CAMEL, was found to be recognized by a CTL clone 1/29 generated by in vitro stimulation of PBMC with autologous IL-2 producing melanoma cells [118]. An HLA-A2 restricted epitope was identified from the gene product translated from an alternative ORF2 of LAGE-1, which is similar to ORF2 in NY-ESO-1 [31]. Interestingly, this CTL clone 1/29 recognized COS-7 transfected with HLA-A2 plus either CAMEL, LAGE-1, or NY-ESO-1 cDNA, suggesting that all three genes are capable of producing the gene products translated from the ORF2 and share identical nucleotide or amino acid sequence in the segment responsible for T cell recognition. This represents another example that antigenic peptides are translated from an alternative ORF. A similar observation was also reported in a mouse system. Gag-specific murine CTL were shown to recognize a peptide generated from ORF2 of the LP-BM5 retrovirus [119]. Despite the fact that many examples of the use of alternative ORFs have been documented, the mechanism by which an alternative ORF is translated remains unclear [120]. The biological significance of these gene products generated from alternative ORFs requires further investigation.

In addition to T cell epitopes identified from the gene products of alternative ORFs, several groups reported that T cell epitopes may also be translated from an intron of incomplete splicing form of mRNA or aberrant mRNA. A cDNA encoding *N*-acetylglucosaminyltransferase V (GnT-V) was isolated using an HLA-A2 restricted CTL clone [121]. A cryptic promoter present within one of the introns of the GnT-V gene appears to be responsible for the generation of an aberrant transcript. The T cell epitope was identified from the gene product of 74 amino acids translated from the intronic transcript of GnT-V. In one study, a gp100 epitope recognized by HLA-A2 restricted CTL cloids was identified from an intron of an incomplete splicing form of the gp100 RNA (gp100–intron 4) [122]. In another recent study, a partially spliced form of TRP-2 (TRP-2–INT2) was isolated by screening a cDNA library using a melanoma-reactive HLA-A68 restricted CTL clone [123]. The T cell epitope recognized by this CTL clone was encoded within the retained intron 2 region. These results suggest that cryptic promoters or aberrant splicing events can result in the translation of the intronic sequences of mRNA and generate proteins or peptides as targets for T cell recognition.

MHC class II restricted tumor antigens recognized by CD4$^+$ T cells

The critical role of CD4$^+$ T cells in antitumor immunity

Much attention has been given to the role of CD8$^+$ T cells in cancer vaccine development. Thus, many tumor antigens recognized by CD8$^+$ T cells have been identified in melanomas as well as in other types of cancers, as discussed above. Although clinical trials using a modified peptide derived from gp100 has shown evidence of therapeutic efficacy for the treatment of patients with metastatic melanoma [11], increasing evidence indicates that optimal cancer vaccines require the participation of both CD4$^+$ and CD8$^+$ T cells [124, 125, 126]. The critical role of tumor specific CD4$^+$ T cells has been demonstrated in the priming of antitumor immunity in several animal models [6, 7, 8, 9]. Other studies have shown that CD4$^+$ T cells are important for the maintenance of CD8$^+$

T cell memory and support $CD8^+$ T cell survival [127]. In addition to its role in antitumor immune responses $CD4^+$ T cells also play a critical role in autoimmune and infectious diseases [128, 129, 130, 131, 132]. Therefore the identification of antigens recognized by $CD4^+$ T cells is important not only for the development of new cancer vaccines but also for a better understanding of the mechanism by which $CD4^+$ T cells regulate host immune responses against cancer.

MHC class II restricted tumor antigens

Many melanoma cell lines naturally express MHC class II molecules and are capable of presenting the antigenic peptide to $CD4^+$ T cells. $CD4^+$ TIL derived from both melanoma and breast cancers have been shown to respond to the autologous tumor cells [133, 134, 135]. Both nonmutated and mutated antigens have been identified.

Nonmutated self-proteins

Tyrosinase. $CD4^+$ melanoma-reactive TIL 1088 recognized an antigenic peptide derived from the tyrosinase protein in an HLA-DR4 restricted fashion [136]. Two distinct peptides QNILLSNAPLGPQFP and DYSYLQDSDPDSFQD recognized by the $CD4^+$ TIL 1088 were subsequently identified (Table 4) [137]. Recently, $CD4^+$ T cells generated from the peripheral blood of a patient with melanoma were shown to recognize synthetic peptides derived from nonmutated tyrosinase. These T cell clones recognized the tyrosinase p386–406 peptide when it was presented by the HLA-DR15 (DRB1*1501) molecule, suggesting that the nonmutated tyrosinase peptides are immunogenic in a melanoma patient [138].

Gp100. Peptide elution from HLA-DR/peptide complexes of a melanoma cell line has led to the identification of a gp100 peptide ($gp100_{44–59}$) [139]. In a subsequent study, tumor specific HLA-DR4-restricted $CD4^+$ T cells was generated from PBMC of a healthy donor by stimulating with HLA-DR transduced Chinese hamster ovary pulsed with the $gp100_{44–59}$ peptide (Table 4). The $CD4^+$ T cells are capable of recognizing $DR4^+/gp100^+$ tumor line FM3, but the T cell reactivity is much enhanced in the presence of antagonistic Fas monoclonal antibody M3 [140]. This raises the possibility that both class I and II peptides from gp100 could be used to achieve an optimal therapeutic effect on the treatment of patient with melanoma.

MAGE-3. MAGE-3-specific $CD4^+$ T cells were recently generated from the PBMC by multiple stimulation in vitro with irradiated PBMC or DC pulsed with synthetic peptides or purified MAGE-3 protein (Table 4) [141, 142]. $CD4^+$ T cells were capable of recognizing the $MAGE\text{-}3_{114–127}$ and $MAGE\text{-}3_{121–134}$ peptides presented by the HLA-DR13 molecule. Although these $CD4^+$ T cells recognized both $HLA\text{-}DR13^+$ EBV B cells and tumor cells transduced with a recombinant retrovirus encoding an Ii-fused MAGE-3, they did not respond to $HLA\text{-}DR13^+/MAGE\text{-}3^+$ tumor cells nor to $HLA\text{-}DR13^+$ EBV B cells transduced with a retrovirus encoding MAGE-3 [141]. These results suggest that the endogenously expressed native MAGE-3 protein could not enter into the MHC class II pathway for the processing and presentation of these two epitopes. However, Manici et al. [142] reported that $CD4^+$ T cells generated from the PBMC of a healthy donor strongly recognized $MAGE\text{-}3_{281–295}$ peptide presented by HLA-DR11. More importantly, the $CD4^+$ T cells recognized $DR11^+/MAGE\text{-}3^+$ tumor cells and exhibited specific cytotoxic activity against the $MAGE\text{-}3^+$ tumor targets, suggesting that the epitope recognized by $CD4^+$ T cells is naturally processed and presented on the cell surface of tumor.

Table 4 MHC class II restricted tumor antigens recognized by $CD4^+$ T cells

Antigens	HLA restrictions	Peptides	References
Nonmutated self-antigens			
Tyrosinase	HLA-DR4	QNILLSNAPLGPQFP (56–70)	137
	HLA-DR4	SYLQDSDPDSFQD (448–462)	137
	HLA-DR15	FLLHHAFVDSIFEQWLQRHRP (386–406)	138
gp100	HLA-DR4	WNRQLYPEWTEAQRLD (44–59)	140
MAGE-3	HLA-DR11	TSYVKVLHHMVKISG (281–295)	142
	HLA-DR13	AELVHFLLLKYRAR (114–127)	141
	HLA-DR13	FLLLKYRAREPVTKAE (119–134)	141
Mutated/fused tumor antigens			
TPI	HLA-DR1	GELIGILNAAKVPAD (23–37)	143, 144
LDFP	HLA-DR1	PVIWRRAPA (312–323)	145
	HLA-DR1	WRRAPAPGA (315–323)	145
CDC27	HLA-DR4	FSWAMDLDPKGA (760–771)	144

Mutated tumor antigens

Several $CD4^+$ T cells established in this laboratory recognize MHC class II^+ autologous tumor cells but do not react with autologous EBV B cells or the known MHC class I restricted tumor antigens. Identification of these new tumor-specific MHC class II restricted antigens is important for the development of effective cancer vaccines.

Triosephosphate isomerase. A mutated triosephosphate isomerase (TPI) was recently identified by a biochemical approach [143]. The point mutation, which resulted in an amino acid substitution of Thr with Ile, created an HLA-DR1-restricted peptide, GELIGILNAAKVPAD, recognized by $CD4^+$ TIL 1558 (Table 4). The Thr/Ile conversion increased T cell reactivity by 5 logs compared to the wild-type peptide [143]. TPI is an enzyme that catalyzes the interconversion of dihydroxyacetone phosphate and glyceraldehyde 3-phosphate, an important step for generation of energy in the glycolytic pathway.

CDC27. One novel genetic approach was recently developed for cloning genes encoding MHC class II restricted tumor antigens. This approach allows the screening of an invariant chain (Ii)–cDNA fusion library in a genetically engineered cell line expressing the essential components of the MHC class II processing pathway [144]. The first antigen identified with this approach is a new mutated human CDC27, an important component of anaphase promoting complex involved in cell cycle regulation, which gives rise to a melanoma target antigen recognized by $CD4^+$ HLA-DR4 restricted TIL 1359 [144]. Although the mutation itself does not constitute a T cell epitope, the Ser/Leu mutation in a putative phosphorylation site allowed a nonmutated peptide within CDC27 to be presented to T cells by MHC class II molecules. Using the same approach, the mutated TPI antigen identified by a biochemical approach was also isolated from a 1558 cDNA library [144], suggesting that the genetic approach is generally applicable to the identification of any antigen recognized by $CD4^+$ T cells.

LDFP. The third antigen identified by this approach is a fusion gene product recognized by HLA-DR1 restricted $CD4^+$ TIL 1363 (Table 4). DNA sequencing analysis indicated that the fusion gene (low-density-lipid receptor fusion protein, LDFP) is generated by a LDLR gene in the 5' end fused to a GDP-L-fucose:β-D-galactoside 2-α-L-fucosyltransferase (FUT) in an antisense orientation in the 3' end [145]. Therefore the fusion gene encodes the first five ligand binding repeats of LDLR in the N-terminus followed by a new polypeptide translated in frame with LDLR from the *FUT* gene in an antisense direction. Chromosomal DNA rearrangements was observed in the 1363mel cell line, and two fusion RNA transcripts were detected in the autologous 1363mel, but not in other cell lines or normal tissues tested. Two overlapping minimal peptides (PVIWRRAPA and WRRAPAPGA) were identified from the C-terminus of the fusion protein [145].

Prospects for cancer therapies

Induction of an anti-self immunity to destroy cancer cells

The identification of tumor antigens recognized by T cells has important implications in the understanding of T cell mediated antitumor activity and provides opportunities for the development of new strategies for cancer vaccines. Because the majority of tumor antigens identified thus far are self-proteins, a potential consequence of active immunization with these cancer peptides is the development of autoimmune disease [146, 147]. Since melanosomal proteins or tissue-specific antigens including tyrosinase, TRP-1, TRP-2, and gp100 are involved in melanin biosynthesis, it is evident that immunotherapy of cancer targeting these antigens may result in depigmentation of skin or vitiligo. Development of vitiligo has been found to be correlated with good prognosis or clinical responses to immunochemotherapy in melanoma patients [43, 148, 149]. The adoptive transfer of autologous TIL along with IL-2 into patients with melanoma results in the objective regression of tumor, and occasionally causes the depigmentation of skin in treated patients [150]. Using tetrameric complexes of MHC class I to identify antigen-specific T cells ex vivo, high frequencies of skin-homing Melan-A/MART-1-specific, A*0201-restricted CTL were found in association with vitiligo in patients [151].

In an animal tumor models the adoptive transfer of CTL clone against murine gp100 resulted in tumor regress of murine B16, but depigmentation of coat color was observed [152]. Recently Houghton and coworkers have demonstrated that passive immunization with a mouse monoclonal antibody (TA99) against gp75/TRP-1 induces protection against and rejection of the $gp75^+$ B16F10 melanoma in syngeneic mice [153]. gp75/TRP-1 could serve as an immune target against melanoma [154, 155]. Immunization of mice with human gp75 protein [156], DNA encoding human gp75/TRP-1 [157], or recombinant vaccinia virus encoding mouse gp75/TRP-1 broke tolerance to murine gp75/TRP-1. Immunity against mouse gp75/TRP-1 provided protection of mice from tumor challenge while manifestations of autoimmunity were observed, characterized by coat depigmentation [157, 158]. Therefore, these studies suggest that induction of immune responses against tissue-specific antigens may represent a new approach for the treatment of cancer.

Targeting of tumor-specific shared antigens for immunotherapy

Tumor antigens including MAGE and NY-ESO-1 are expressed in several types of cancer but not in normal human tissues with the exception of testis [25, 31, 159]. Because testis is seen as an immune privileged site, targeting these antigens provide a more specific antitumor im-

munity against cancer. More importantly, strategies developed for one type of cancer could be used for different types of cancer since these antigens are expressed in various types of cancers, including melanoma, breast, prostate, and lung cancers. The utility of this class of antigens is being tested in treating patient with melanoma [160].

Vaccine strategies

Peptide-based vaccines. Active immunotherapy involves the direct immunization of cancer patients with cancer antigens in an attempt to boost immune responses against the tumor. The immunodominant peptides derived from tumor antigens could readily be synthesized in vitro and used for immunization either alone or in a form intended to improve their immunogenicity, such as in combination with adjuvant, linkage to lipids/liposomes or helper peptides, or pulsed onto APC. Modification of the immunodominant peptides to improve binding efficiency to MHC antigens can potentially increase immunogenicity and induce stronger antitumor activity [161]. Clinical studies have indicated that peptide vaccines using a modified gp100 peptide combined with high dose of IL-2 result in a 42% of clinical response rate in HLA-A2$^+$ melanoma patients [11]. Since multiple tumor antigens and multiple epitopes have been identified from melanoma, it is likely that the use of multiple epitope peptides would enhance an antitumor activity.

Dendritic cell-based vaccines. Dendritic cells are potent APC and play an important role in priming and maintenance of an antitumor immunity [162]. DC pulsed with peptides or proteins and infected with recombinant viruses encoding tumor antigens have been shown to induce potent antitumor immunity [163, 164, 165, 166]. Several clinical trials using DC pulsed with peptides from tumor antigens have been conducted [166]. It has recently been reported that patients treated with DC pulsed peptides or tumor lysates resulted in an objective clinical regression of tumor in 5 of 16 patients evaluated.

Recombinant viruses/nucleotide acid based vaccines. One of the most effective cancer vaccines may involve the incorporation of genes encoding tumor antigens into recombinant plasmid or viruses such as vaccinia, fowlpox, or adenovirus. Because tumor cells transduced with genes encoding cytokines or costimulatory molecules have been shown to elicit in vivo antitumor activity, the combination of cancer antigen genes with other genes encoding cytokines such as IL-2, costimulatory molecules such as B7.1 may enhance the immune response following viral infection. Alternatively, cancer vaccines using recombinant virus encoding tumor antigens can be enhanced by the exogenous administration of immunostimulatory cytokines. The major problem associated with recombinant viruses encoding tumor antigens is that patients develop strong antibody responses against the recombinant viruses, resulting in inefficient or low infection activity [12].

DC infected with recombinant adenoviruses may overcome this problem [167, 168]. “Naked” DNA vaccines are an alternative to the use of recombinant viruses [169]. Self-replication RNA viruses encoding a model antigen has recently been shown to produce a strong antitumor activity [170].

Since optimal vaccines require both CD4$^+$ and CD8$^+$ T cells, strategies using combination of MHC class I and class II tumor antigens may aid the development of effective cancer vaccines [9, 132]. Clinical trials using both MHC class I and II restricted tumor antigens are being conducted at the National Cancer Institute. Combination therapy using peptides/proteins, DC, and recombinant viruses/DNA may be a next wave of cancer immunotherapy.

References

1. Rosenberg SA, Spiess P, Lafreniere R (1986) A new approach to the adoptive immunotherapy of cancer with tumor-infiltrating lymphocytes. Science 233:1318–1321
2. Kast WM, Offringa R, Peters PJ, Voordouw AC, Meloen RH, Van der Eb AJ, Melief CJM (1989) Eradication of adenovirus E1-induced tumors by E1 A specific cytotoxic T lymphocytes. Cell 59:603–614
3. Greenberg PD (1991) Adoptive T cell therapy of tumors: mechanisms operative in the recognition and elimination of tumor cells. Adv Immunol 49:281–355
4. Rosenberg SA, Packard BS, Aebersold PM, Solomon D, Topalian SL, Toy ST, Simon P, Lotze MT, Yang JC, Seipp CA, Simpson C, Carter C, Bock S, Schwartzentruber D, Wei JP, White DE (1988) Use of tumor infiltrating lymphocytes and interleukin-2 in the immunotherapy of patients with metastatic melanoma. Preliminary report. N Engl J Med 319:1676–1680
5. Rosenberg SA (1992) The immunotherapy and gene therapy of cancer. J Clin Oncol 10:180–199
6. Greenberg PD, Cheever MA, Fefer A (1981) Eradication of disseminated murine leukemia by chemoimmunotherapy with cyclophosphamide and adoptively transferred immune syngeneic Lyt-1+2-lymphocytes. J Exp Med 154:952–963
7. Schild HJ, Kyewski B, Von Hoegen P, Schirrmacher V (1987) CD4+ helper T cells are required for resistance to a highly metastatic murine tumor. Eur J Immunol 17:1863–1866
8. Romerdahl CA, Kripke ML (1988) Role of helper T-lymphocytes in rejection of UV-induced murine skin cancers. Cancer Res 48:2325–2328
9. Hung K, Hayashi R, Lafond-Walker A, Lowenstein C, Pardoll D, Levitsky H (1998) The central role of CD4(+) T cells in the antitumor immune response. J Exp Med 188:2357–2368
10. Ossendorp F, Mengede E, Camps M, Filius R, Melief CJ (1998) Specific T helper cell requirement for optimal induction of cytotoxic T lymphocytes against major histocompatibility complex class II negative tumors. J Exp Med 187:693–702
11. Rosenberg SA, Yang JC, Schwartzentruber DJ, Hwu P, Marincola FM, Topalian SL, Restifo NP, Dudley ME, Schwarz SL, Spiess PJ, Wunderlich JR, Parkhurst MR, Kawakami Y, Seipp CA, Jan RN, Einhorn RN, White DE (1998) Immunologic and therapeutic evaluation of a synthetic tumor-associated peptide vaccine for the treatment of patients with metastatic melanoma. Nat Med 4:321–327
12. Rosenberg SA, Zhai Y, Yang JC, Schwartzentruber DJ, Hwu P, Marincola FM, Topalian SL, Restifo NP, Seipp CA, Einhorn JH, Roberts B, White DE (1998) Immunizing patients with metastatic melanoma using recombinant adenoviruses encoding MART-1 or gp100 melanoma antigens. J Natl Cancer Inst 90:1894–1900

13. Van Den Eynde B, Lethë B, Van Pel A, De Plaen E, Boon T (1991) The gene coding for a major tumor rejection antigen of tumor P815 is identical to the normal gene of syngeneic DBA/2 mice. J Exp Med 173:1373–1384
14. Brichard V, Van Pel A, Wölfel T, Wölfel C, De Plaen E, Lethë B, Coulie P, Boon T (1993) The tyrosinase gene codes for an antigen recognized by autologous cytolytic T lymphocytes on HLA-A2 melanomas. J Exp Med 178:489–495
15. Coulie PG, Brichard V, Van Pel A, Wolfel T, Schneider J, Traversari C, Mattei S, De Plaen ED, Lurquin C, Szikora J-P, Reauld J-C, Boon T (1994) A new gene coding for a differentiation antigen recognized by autologous cytolytic T lymphocytes on HLA-A2 melanomas. J Exp Med 180:35–42
16. Wang R-F, Robbins PF, Kawakami Y, Kang XQ, Rosenberg SA (1995) Identification of a gene encoding a melanoma tumor antigen recognized by HLA-A31-restricted tumor-infiltrating lymphocytes. J Exp Med 181:799–804
17. Wang R-F, Wang X, Johnston SL, Zeng G, Robbins PF, Rosenberg SA (1998) Development of a retrovirus-based complementary DNA expression system for the cloning of tumor antigens. Cancer Res 58:3519–3525
18. Cox AL, Skipper J, Cehn Y, Henderson RA, Darrow TL, Shabanowitz J, Engelhard VH, Hunt DF, Slingluff CL (1994) Identification of a peptide recognized by five melanoma-specific human cytotoxic T cell lines. Science 264:716–719
19. Blake J, Johnston JV, Hellstrom KE, Marqardt H, Chen L (1996) Use of combinatorial peptide libraries to construct functional mimics of tumor epitopes recognized by MHC class I-restricted cytolytic T lymphocytes. J Exp Med 184:121–130
20. Cheever MA, Disis ML, Bernhard H, Gralow JR, Hand SL, Huseby ES, Qin HL, Takahashi M, Chen W (1995) Immunity to oncogenic proteins. Immunol Rev 145:33–59
21. Fisk B, Blevins TL, Wharton JT, Ioannides CG (1995) Identification of an immunodominant peptide of HER-2/neu protooncogene recognized by ovarian tumor-specific cytotoxic T lymphocyte lines. J Exp Med 181:2109–2117
22. Sahin U, Tureci O, Pfreundschuh M (1997) Serological identification of human tumor antigens. Curr Opin Immunol 9:709–716
23. Old LJ, Chen YT (1998) New paths in human cancer serology. J Exp Med 187:1163–1167
24. Sahin U, Tureci O, Schmitt H, Cochlovius B, Johannes T, Schmits R, Stenner F, Luo G, Schobert I, Pfreundschuh M (1995) Human neoplasms elicit multiple immune responses in the autologous host. Proc Natl Acad Sci USA 92:11810–11813
25. Chen YT, Scanlan MJ, Sahin U, Tureci O, Gure AO, Tsang S, Williamson B, Stockert E, Pfreundschuh M, Old LJ (1997) A testicular antigen aberrantly expressed in human cancers detected by autologous antibody screening. Proc Natl Acad Sci USA 94:1914–1918
26. Tureci O, Sahin U, Schobert I, Koslowski M, Scmitt H, Schild HJ, Stenner F, Seitz G, Rammensee HG, Pfreundschuh M (1996) The SSX-2 gene, which is involved in the t(X;18) translocation of synovial sarcomas, codes for the human tumor antigen HOM-MEL-40. Cancer Res 56:4766–4772
27. Tureci O, Sahin U, Zwick C, Koslowski M, Seitz G, Pfreundschuh M (1998) Identification of a meiosis-specific protein as a member of the class of cancer-testis antigens. Proc Natl Acad Sci USA 95:5211–5216
28. Chen YT, Gure AO, Tsang S, Stockert E, Jager E, Knuth A, Old LJ (1998) Identification of multiple cancer/testis antigens by allogeneic antibody screening of a melanoma cell line library. Proc Natl Acad Sci U S A 95:6919–6923
29. Van der Bruggen P, Traversari C, Chomez P, Lurquin C, DePlaen E, Van Den Eynde B, Knuth A, Boon T (1991) A gene encoding an antigen recognized by cytolytic T lymphocytes on a human melanoma. Science 254:1643–1647
30. Jager E, Chen YT, Drijfhout JW, Karbach J, Ringhoffer M, Jager D, Arand M, Wada H, Noguchi Y, Stockert E, Old LJ, Knuth A (1998) Simultaneous humoral and cellular immune response against cancer-testis antigen NY-ESO-1: definition of human histocompatibility leukocyte antigen (HLA)-A2-binding peptide epitopes. J Exp Med 187:265–270
31. Wang R-F, Johnston SL, Zeng G, Schwartzentruber DJ, Rosenberg SA (1998) A breast and melanoma-shared tumor antigen: T cell responses to antigenic peptides translated from different open reading frames. J Immunol 161:3596–3606
32. Jung S, Schluesener HJ (1991) Human T lymphocytes recognize a peptide of single point-mutated, oncogenic ras proteins. J Exp Med 173:273–276
33. Skipper J, Stauss HJ (1993) Identification of two cytotoxic T lymphocyte-recognized epitopes in the Ras protein. J Exp Med 177:1493–1498
34. Celis E, Tsai V, Crimi C, DeMars R, Wentworth PA, Chesnut RW, Grey HM, Sette A, Serra HM (1994) Induction of anti-tumor cytotoxic T lymphocytes in normal humans using primary cultures and synthetic peptide epitopes. Proc Natl Acad Sci USA 91:2105–2109
35. Zaks TZ, S. A. Rosenberg (1998) Immunization with a peptide epitope (p369–377) from HER-2/neu leads to peptide specific cytotoxic T lymphocytes which fail to recognize HER-2/neu+ tumors. Cancer Res 58:4902–4908
36. Valmori D, Gileadi U, Servis C, Dunbar PR, Cerottini JC, Romero P, Cerundolo V, Levy F (1999) Modulation of proteasomal activity required for the generation of a cytotoxic T lymphocyte-defined peptide derived from the tumor antigen MAGE-3. J Exp Med 189:895–906
37. Theobald M, Ruppert T, Kuckelkorn U, Hernandez J, Haussler A, Ferreira EA, Liewer U, Biggs J, Levine AJ, Huber C, Koszinowski UH, Kloetzel PM, Sherman LA (1998) The sequence alteration associated with a mutational hotspot in p53 protects cells from lysis by cytotoxic T lymphocytes specific for a flanking peptide epitope. J Exp Med 188:1017–1028
38. Rammensee HG, Friede T, Stevanoviic S (1995) MHC ligands and peptide motifs: first listing. Immunogenetics 41:178–228
39. Theobald M, Biggs J, Dittmer D, Levine AJ, Sherman LA (1995) Targeting p53 as a general tumor antigen. Proc Natl Acad Sci USA 92:11993–11997
40. Theobald M, Biggs J, Hernandez J, Lustgarten J, Labadie C, Sherman LA (1997) Tolerance to p53 by A2.1-restricted cytotoxic T lymphocytes. J Exp Med 185:833–841
41. Bouchard B, Fuller BB, Vijayasaradhi S, Houghton AN (1989) Induction of pigmentation in mouse fibroblasts by expression of human tyrosinase cDNA. J Exp Med 169:2029–2042
42. Wolfel T, Van Pel A, Brichard V, Schneider J, Seliger B, Meyer Zum Buschenfelde K-H, Boon T (1994) Two tyrosinase nonapeptides recognized on HLA-A2 melanomas by autologous cytolytic T lymphocytes. Eur J Immunol 24:759–764
43. Visseren MJ, Van Elsas A, van der Voort EI, Ressing ME, Kast WM, Schrier PI, Melief CJ (1995) CTL specific for the tyrosinase autoantigen can be induced from healthy donor blood to lyse melanoma cells. J Immunol 154:3991–3998
44. Robbins PF, El-Gamil M, Kawakami Y, Stevens E, Yannelli J, Rosenberg SA (1994) Recognition of tyrosinase by tumor infiltrating lymphocytes from a patient responding to immunotherapy. Cancer Res 54:3124–3126
45. Kang X-Q, Kawakami Y, Sakaguchi K, El-Gamil M, Wang R-F, Yannelli JR, Appella E, Rosenberg SA, Robbins PF (1995) Identification of a tyrosinase epitope recognized by HLA-A24 restricted tumor-infiltrating lymphocytes. J Immunol 155: 1343–1348
46. Brichard VG, Herman J, Van Pel A, Wildmann C, Gaugler B, Wolfel T, Boon T, Lethe B (1996) A tyrosinase nonpeptide presented by HLA-B44 is recognized on a human melanoma by autologous cytolytic T lymphocytes. Eur J Immunol 26:224–230
47. Skipper JC, Hendrickson RC, Gulden PH, Brichard V, Van Pel A, Chen Y, Shabanowitz J, Wolfel T, Slingluff CL, Boon T, Hunt D (1996) An HLA-A2-restricted tyrosinase antigen on melanoma cells results from posttranslational modification and suggests a novel pathway for processing of membrane proteins. J Exp Med 183:527–534
48. Kawakami Y, Eliyahu S, Delgaldo CH, Robbins PF, Rivoltini L, Topalian SL, Miki T, Rosenberg SA (1994) Cloning of the gene coding for a shared human melanoma antigen recognized by autologous T cells infiltrating into tumor. Proc Natl Acad Sci USA 91:3515–3519

49. Kawakami Y, Eliyahu S, Sakaguchi K, Robbins PF, Rivoltini L, Yannelli JB, Appella E, Rosenberg SA (1994) Identification of the immunodominant peptides of the MART-1 human melanoma antigen recognized by the majority of HLA-A2 restricted tumor infiltrating lymphocytes. J Exp Med 180: 347–352
50. Sensi M, Traversari C, Radrizzani M, Salvi S, Maccalli C, Mortarini R, Rivoltini L, Farina C, Nicolini G, Wolfel T, Brichard V, Boon T, Bordignon C, Anichini A, Parmiani G (1995) Cytotoxic T lymphocyte clones from different patients display limited T-cell receptors variable gene usage in HLA-A2 restricted recognition of Melan/Mart-1 melanoma antigen. Proc Natl Acad Sci USA 92:5674–5678
51. Valmori D, Fonteneau JF, Lizana CM, Gervois N, Lienard D, Rimoldi D, Jongeneel V, Jotereau F, Cerottini JC, Romero P (1998) Enhanced generation of specific tumor-reactive CTL in vitro by selected Melan-A/MART-1 immunodominant peptide analogues. J Immunol 160:1750–1758
52. Schneider J, Brichard V, Boon T, Meyer zum Buschenfelde KH, Wolfel T (1998) Overlapping peptides of melanocyte differentiation antigen Melan-A/MART-1 recognized by autologous cytolytic T lymphocytes in association with HLA-B45.1 and HLA-A2.1. Int J Cancer 75:451–458
53. Loftus DJ, Castelli C, Clay TM, Squarcina P, Marincola FM, Nishimura MI, Parmiani G, Appella E, Rivoltini L (1996) Identification of epitope mimics recognized by CTL reactive to the melanoma/melanocyte-derived peptide MART-1 27–35. J Exp Med 184:647–657
54. Kawakami Y, Eliyahu S, Jennings C, Sakaguchi K, Kang X, Southwood S, Robbins PF, Sette A, Appella E, Rosenberg SA (1995) Recognition of multiple epitopes in the human melanoma antigen gp100 by tumor-infiltrating T lymphocytes associated with in vivo tumor regression. J Immunol 154:3961–3968
55. Adema GJ, de Boer AJ, van't Hullenaar R, Denijn M, Ruiter DJ, Vogel AM, Figdor CG (1993) Melanocyte lineage-specific antigens recognized by monoclonal antibodies NK1-beteb, HMB-50, and HMB-45 are encoded by a single cDNA. Am J Pathol 143:1579–1585
56. Bakker ABH, Schreurs MWJ, de Boer AJ, Kawakami Y, Rosenberg SA, Adema GJ, Figdor CG (1994) Melanocyte lineage-specific antigen gp100 is recognized by melanocyte-derived tumor-infiltrating lymphocytes. J Exp Med 179:1005–1009
57. Kawakami Y, Eliyahu S, Delgado CH, Robbins PF, Sakaguchi K, Appella E, Yannelli JR, Adema GJ, Miki T, Rosenberg SA (1994) Identification of a human melanoma antigen recognized by tumor infiltrating lymphocytes associated with in vivo tumor rejection. Proc Natl Acad Sci USA 91:6458–6462
58. Bakker A, Schreurs M, Tafazzul G, de Boer A, Kawakami Y, Adema G, Figdor C (1995) Identification of a novel peptide derived from the melanocyte-specific gp100 antigen as the dominant epitope recognized by an HLA-A2.1-restricted anti-melanoma CTL line. Int J Cancer 62:97–102
59. Kawakami Y, Robbins PF, Wang X, Tupesis JP, Parkhurst MR, Kang X, Sakaguchi K, Appella E, Rosenberg SA (1998) Identification of new melanoma epitopes on melanosomal proteins recognized by tumor infiltrating T lymphocytes restricted by HLA-A1, -A2, and -A3 alleles. J Immunol 161:6985–6992
60. Skipper JCA, Kittlesen DJ, Hendrickson RA, Deacon DD, Harthun NL, Wagner SN, Hunt DF, Engelhard VH, Slingluff CL (1996) Shared epitopes for HLA-A3-restricted melanoma-reactive human CTL include a naturally processed epitope from Pmel-17/gp100. J Immunol 157:5027–5033
61. Mattes MJ, Thomson TM, Old LJ, Lloyd KO (1983) A pigmentation-associated, differentiation antigen of human melanoma defined by a precipitating antibody in human serum. Int J Cancer 32:717–721
62. Thomson TM, Mattes JM, Roux L, Old LJ, Lloyd KO (1985) Pigmentation-associated glycoprotein of human melanoma and melanocytes: definition with a mouse monoclonal antibody. J Invest Dermatol 85:169–174
63. Thomson TM, Real FX, Murakami S, Cordon-Cardo C, Old LJ, Houghton AN (1988) Differentiation antigens of melanocytes and melanoma: analysis of melanosome and cell surface markers of human pigmented cells with monoclonal antibodies. J Invest Dermatol 90:459–466
64. Jimenez-Cervantes C, Solano F, Kobayashi T, Urabe K, Hearing VJ, Lozano JA, Garcia-Borron JC (1994) A new enzymatic function in the melanogenic pathway. J Biol Chem 269:17993–18001
65. Wang R-F, Appella E, Kawakami Y, Kang X, Rosenberg SA (1996) Identification of TRP-2 as a human tumor antigen recognized by cytotoxic T lymphocytes. J Exp Med 184:2207–2216
66. Bloom MB, Perry-Lalley D, Robbins PF, Li Y, El-Gamil M, Rosenberg SA, Yang JC (1997) Identification of tyrosinase-related protein 2 as a tumor rejection antigen for the B16 melanoma. J Exp Med 185:453–460
67. Zeh HJ, 3rd, Perry-Lalley D, Dudley ME, Rosenberg SA, Yang JC (1999) High avidity CTLs for two self-antigens demonstrate superior in vitro and in vivo antitumor efficacy. J Immunol 162:989–994
68. Sidney J, Grey HM, Kubo RT, Sette A (1996) Practical, biochemical and evolutionary implications of the discovery of HLA class I supermotifs. Immunol Today 17:261–266
69. Sidney J, Grey HM, Southwood S, Celis E, Wentworth PA, Guercio MFD, Kubo R, Chesnut RW, Sette A (1996) Definition of an HLA-A3-like supermotif demonstrates the overlapping peptide-binding repertoires of common HLA molecules. Human Immunology 45:79–93
70. Wang R-F, Johnston S, Southwood S, Sette A, Rosenberg SA (1998) Recognition of an antigenic peptide derived from TRP-2 by cytotoxic T lymphocytes in the context of HLA-A31 and -A33. J Immunol 160:890–897
71. Parkhurst MR, Fitzgerald EB, Southwood S, Sette A, Rosenberg SA, Kawakami Y (1998) Identification of a shared HLA-A*0201-restricted T-cell epitope from the melanoma antigen tyrosinase-related protein 2 (TRP2). Cancer Res 58:4895–4901
72. Traversari C, Van der Bruggen P, Luescher IF, Lurquin C, Chomez P, Van Pel A, De Plaen E, Amar-Costesec A, Boon T (1992) A nonapeptide encoded by human gene MAGE-1 is recognized on HLA-A1 by cytolytic T lymphocytes directed against tumor antigen MZ2-E. J Exp Med 176:1453–1457
73. Van der Bruggen P, Szikora J-P, Boel P, Wildmann C, Somville M, Sensi M, Boon T (1994) Autologous cytolytic T lymphocytes recognize a MAGE-1 nonapeptide on melanomas expressing Cw1601. Eur J Immunol 24:2134–2140
74. Gaugler B, Van Den Eynde B, Van der Bruggen P, Romero P, Gaforio JJ, De Plaen E, Lethe B, Brasseur F, Boon T (1994) Human gene MAGE-3 codes for an antigen recognized on a melanoma by autologous cytolytic T lymphocytes. J Exp Med 179:921–930
75. Van der Bruggen P, Bastin J, Gajewski T, Coulie PG, Boel P, de Smet C, Traversari C, Townsend A, Boon T (1994) A peptide encoded by human gene MAGE-3 and presented by HLA-A2 induces cytolytic T lymphocytes that recognize tumor cells expressing MAGE-3. Eur J Immunol 24:3038–3043
76. Herman J, van der Bruggen P, Luescher IF, Mandruzzato S, Romero P, Thonnard J, Fleischhauer K, Boon T, Coulie PG (1996) A peptide encoded by the human MAGE3 gene and presented by HLA-B44 induces cytolytic T lymphocytes that recognize tumor cells expressing MAGE3. Immunogenetics 43:377–383
77. Boel P, Wildmann C, Sensi ML, Brasseur R, Renauld JC, Coulie P, Boon T, Van der Bruggen P (1995) BAGE: a new gene encoding an antigen recognized on human melanomas by cytolytic T lymphocytes. Immunity 2:167–175
78. Van Den Eynde B, Peeters O, De Backer O, Gaugler B, Lucas S, Boon T (1995) A new family of genes coding for an antigen recognized by autologous cytolytic T lymphocytes on a human melanoma. J Exp Med 182:689–698
79. Brinkmann U, Vasmatzis G, Lee B, Pastan I (1999) Novel genes in the PAGE and GAGE family of tumor antigens found by homology walking in the dbEST database. Cancer Res 59:1445–1448

80. Brinkmann U, Vasmatzis G, Lee B, Yerushalmi N, Essand M, Pastan I (1998) PAGE-1, an X chromosome-linked GAGE-like gene that is expressed in normal and neoplastic prostate, testis, and uterus. Proc Natl Acad Sci U S A 95:10757–10762
81. Chen ME, Lin SH, Chung LW, Sikes RA (1998) Isolation and characterization of PAGE-1 and GAGE-7. New genes expressed in the LNCaP prostate cancer progression model that share homology with melanoma-associated antigens. J Biol Chem 273:17618–17625
82. De Backer O, Arden KC, Boretti M, Vantomme V, De Smet C, Czekay S, Viars CS, De Plaen E, Brasseur F, Chomez P, Van den Eynde B, Boon T, van der Bruggen P (1999) Characterization of the GAGE genes that are expressed in various human cancers and in normal testis. Cancer Res 59:3157–3165
83. Lee L, Wang R-F, Wang X, Mixon A, Johnson BE, Rosenberg SA, Schrump DS (1998) NY-ESO-1 may be a potential target for lung cancer immunotherapy. Cancer J Sci Am 5:20–25
84. Stockert E, Jager E, Chen YT, Scanlan MJ, Gout I, Karbach J, Arand M, Knuth A, Old LJ (1998) A survey of the humoral immune response of cancer patients to a panel of human tumor antigens. J Exp Med 187:1349–1354
85. Gaugler B, Brouwenstijn N, Vantomme V, Szikora JP, Van der Spek CW, Patard JJ, Boon T, Schrier P, Van den Eynde BJ (1996) A new gene coding for an antigen recognized by autologous cytolytic T lymphocytes on a human renal carcinoma. Immunogenetics 44:323–330
86. Van Elsas A, Nijman HW, Van der Minne CE, Mourer JS, Kast WM, Melief CJ, Schrier PI (1995) Induction and characterization of cytotoxic T-lymphocytes recognizing a mutated p21ras peptide presented by HLA-A*0201. Int J Cancer 61:389–396
87. Houbiers JGA, Nijman HW, van der Burg SH, Drijfhout JW, Kenemans P, van de Velde CJH, Brand A, Momburg F, Kast WM, Melief CJ M (1993) In vitro induction of human cytotoxic T lymphocyte responses against peptides of mutant and wild-type p53. Eur J Immunol 23:2072–2077
88. Nijman HW, Houbiers JGA, Vierboom MPM, van der Burg SH, Drijfhout JW, D'Amaro J, Kenemans P, Melief CJM, Kast WM (1993) Identification of peptide sequences that potentially trigger HLA-A2.1-restricted cytotoxic T lymphocytes. Eur J Immunol 23:1215–1219
89. Nijman HW, van der Burg SH, Vierboom MP, Houbiers JG, Kast WM, Melief CJ (1994) p53, a potential target for tumor-directed T cells. Immunol Lett 40:171–178
90. Coulie PG, Lehmann F, Lethe B, Herman J, Lurquin C, Andrawiss M, Boon T (1995) A mutated intron sequence codes for an antigenic peptide recognized by cytolytic T lymphocytes on a human melanoma. Proc Natl Acad Sci USA 92:7976–7980
91. Wolfel T, Hauer M, Schneider J, Serrano M, Wolfel C, Klehmann-Hieb E, De Plaen E, Hankeln T, Meyer Zum Buschenfelde K-H, Beach D (1995) A p16INK4a-insensitive CDK4 mutant targeted by cytolytic T lymphocytes in a human melanoma. Science 269:1281–1284
92. Sherr CJ, Roberts JM (1995) Inhibitors of mammalian G1 cyclin-dependent kinases. Genes & Development 9:1149–1163
93. Robbins PF, El-Gamil M, Li YF, Kawakami Y, Loftus D, Appella E, Rosenberg SA (1996) A mutated β-catenin gene encodes a melanoma-specific antigen recognized by tumor infiltrating lymphocytes. J Exp Med 183:1185–1192
94. Grunwald GB (1993) The structural and functional analysis of cadherin calcium-dependent cell adhesion molecules. Curr Opin Cell Biol 5:797–805
95. Hoschuetzky H, Aberle H, Kemler R (1994) β-catenin mediates the interaction of the cadherin-catenin complex with epidermal growth factor receptor. J Cell Biol 127:1375–1380
96. Becker K-F, Atkinson MJ, Reich U, Becker I, Nekarda H, Siewert JR, Hofler H (1994) E-cadherin gene mutations provide clues to diffuse type gastric carcinomas. Cancer Res 54:3845–3852
97. Kawanishi J, Kato J, Sasaki K, Fujii S, Watanabe M, Niitsu Y (1995) Loss of E-cadherin-dependent cell-cell adhesion due to mutation of the β-catenin gene in a human cancer cell line, HSC-39. Mol Cell Biol 15:1175–1181
98. Korinek V, Baker N, Morin PJ, van Wichen D, de Weger R, Kinzler KW, Vogelstein B, Clevers H (1997) Constitutive transcriptional activation by a beta-catenin-Tcf complex in APC–/– colon carcinoma. Science 275:1784–1787
99. Morin PJ, Sparks AB, Korinek V, Barker N, Clevers H, Vogelstein B, Kinzler KW (1997) Activation of beta-catenin-Tcf signaling in colon cancer by mutations in beta-catenin or APC. Science 275:1787–1790
100. Rubinfeld B, Robbins P, El-Gamil M, Albert I, Porfiri E, Polakis P (1997) Stabilization of beta-catenin by genetic defects in melanoma cell lines. Science 275:1790–1792
101. Mandruzzato S, Brasseur F, Andry G, Boon T, van der Bruggen P (1997) A CASP-8 mutation recognized by cytolytic T lymphocytes on a human head and neck carcinoma. J Exp Med 186:785–793
102. Ioannides CG, Fisk B, Jerome KR, Irimura T, Wharton JT, Finn OJ (1993) Cytotoxic T cells from ovarian malignant tumors can recognize polymorphic epithelial mucin core peptides. J Immunol 151:3693–3703
103. Magarian-Blander J, Domenech N, Finn OJ (1993) Specific and effective T-cell recognition of cells transfected with a truncated human mucin cDNA. Ann NY Acad Sci 690: 231–243
104. Finn OJ, Jerome KR, Henderson RA, Pecher G, Domenech N, Magarian-Blander B-B. SM (1995) MUC-1 epithelial tumor mucin-based immunity and cancer vaccines. Immunol Rev 145:61–89
105. Apostolopoulos V, Karanikas V, Haurum JS, McKenzie IF (1997) Induction of HLA-A2-restricted CTLs to the mucin 1 human breast cancer antigen. J Immunol 159:5211–5218
106. Domenech N, Henderson RA, Finn OJ (1995) Identification of an HLA-A11-restricted epitope from the tandem repeat domain of the epithelial tumor antigen mucin. J Immunol 155:4766–4774
107. Apostolopoulos V, Lofthouse SA, Popovski V, Chelvanayagam G, Sandrin MS, McKenzie IF (1998) Peptide mimics of a tumor antigen induce functional cytotoxic T cells. Nat Biotechnol 16:276–280
108. Ioannides CG, Fisk B, Pollack MS, Frazier ML, Wharton JT, Freedman RS (1993) Cytotoxic T-cell clones isolated from ovarian tumour infiltrating lymphocytes recognize common determinants on nonovarian tumour clones. Scand J Immunol 37:413–424
109. Yoshino I, Peoples GE, Geoedegebauure PS, Mazizrz R, Eberlein TJ (1994) Association of HER2/neu expression with sensitivity to tumor-specific CTL in human ovarian cancer. J Immunol 152:2393–2400
110. Disis ML, CAlenoff E, McLaughlin G, Murphy AE, Chen W, Groner B, Jeschke M, Lydon N, McGlynn E, Livingston RB, Moe R, Cheever MA (1994) Existent T cell and antibody immunity to HER-2/neu protein in patients with breast cancer. Cancer Res 54:16–20
111. Peoples GE, Goedegebuure PS, Smith R, Linehan DC, Yoshino I, Eberlein TJ (1995) Breast and ovarian cancer-specific cytotoxic T lymphocytes recognize the same HER2/neu-deroed peptide. Proc Natl Acad Sci USA 92:432–436
112. Linehan DC, Goedegebuure PS, Peoples GE, Rogers SO, Eberlein TJ (1995) Tumor-specific and HLA-A2-restricted cytolysis by tumor-associated lymphocytes in human metastatic breast cancer. J Immunol 155:4486–4491
113. Kawashima I, Tsai V, Southwood S, Takesako K, Sette A, Celis E (1999) Identification of HLA-A3-restricted cytotoxic T lymphocyte epitopes from carcinoembryonic antigen and HER-2/neu by primary in vitro immunization with peptide-pulsed dendritic cells. Cancer Res 59:431–435
114. Wang R-F, Parkhurst MR, Kawakami Y, Robbins PF, Rosenberg SA (1996) Utilization of an alternative open reading frame of a normal gene in generating a novel human cancer antigen. J Exp Med 183:1131–1140
115. Spiropoulou CF, Nichol ST (1993) A small highly basic protein is encoded in overlapping frame within the P gene of vesicular stomatitis virus. J Virol 67:3103–3110

116. Schwartz S, Felber BK, Pavlakis GN (1992) Mechanism of translation of monocistronic and multicistronic human immunodeficiency virus type 1 mRNAs. Mol Cell Biol 12:207–219
117. Lethe B, Lucas S, Michaux L, De Smet C, Godelaine D, Serrano A, De Plaen E, Boon T (1998) LAGE-1, a new gene with tumor specificity. Int J Cancer 76:903–908
118. Aarnoudse CA, van den Doel PB, Heemskerk B, Schrier PI (1999) Interleukin-2-induced, melanoma-specific T cells recognize CAMEL, an unexpected translation product of LAGE-1. Int J Cancer 82:442–448
119. Mayrand SM, Schwarz DA, Green WR (1998) An alternative translational reading frame encodes an immunodominant retroviral CTL determinant expressed by an immunodeficiency-causing retrovirus. J Immunol 160:39–50
120. Mayrand SM, Green WR (1998) Non-traditionally derived CTL epitopes: exceptions that prove the rules? Immunol Today 19:551–556
121. Guilloux Y, Lucas L, Brichard VG, Van Pel A, Viret C, De Plaen E, Brasseur F, Lethe B, Jotereau F, Boon T (1996) A peptide recognized by human cytolytic T lymphocytes on HLA-A2 melanomas is encoded by an intron sequence of the *N*-acetylglucosaminyltransferase V gene. J Exp Med 183: 1173–1183
122. Robbins P, El-Gamil M, Li YF, Fitzgerald E, Kawakami Y, Rosenberg SA (1997) The intronic region of an incompletely spliced gp100 gene transcript encodes an epitope recognized by melanoma-reactive tumor-infiltrating lymphocytes. J Immunol 159:303–308
123. Lupetti R, Pisarra P, Verrecchia A, Farina C, Nicolini G, Anichini A, Bordignon C, Sensi M, Parmiani G, Traversari C (1998) Translation of a retained intron in tyrosinase-related protein (TRP) 2 mRNA generates a new cytotoxic T lymphocyte (CTL)-defined and shared human melanoma antigen not expressed in normal cells of the melanocytic lineage. J Exp Med 188:1005–1016
124. Ostrand-Rosenberg S (1994) Tumor immunotherapy: the tumor cell as an antigen-presenting cell. Curr Opin Immunol 6:722–727
125. Pardoll DM, Topalian SL (1998) The role of CD4(+) T cell responses in antitumor immunity. Curr Opin Immunol 10: 588–594
126. Toes RE, Ossendorp F, Offringa R, Melief CJ (1999) CD4 T Cells and their role in antitumor immune responses. J Exp Med 189:753–756
127. Frasca L, Piazza C, Piccolella E (1998) CD4+ T cells orchestrate both amplification and deletion of CD8+ T cells. Crit Rev Immunol 18:569–594
128. Tisch R, McDevitt H (1996) Insulin-dependent diabetes mellitus. Cell 85:291–297
129. O'Garra A, Steinman L, Gijbels K (1997) CD4+ T-cell subsets in autoimmunity. Curr Opin Immunol 9:872–883
130. Bona CA, Casares S, Brumeanu TD (1998) Towards development of T-cell vaccines. Immunol Today 19:126–133
131. Riddell SR, Greenberg PD (1995) Principles for adoptive T cell therapy of human viral diseases. Annu Rev Immunol 13:545–586
132. Kalams SA, Walker BD (1998) The critical need for CD4 help in maintaining effective cytotoxic T lymphocyte responses. J Exp Med 188:2199–2204
133. Markus NR, Rosenberg SA, Topalian SL (1995) Analysis of cytokine secretion by melanoma-specific CD4+ T lymphocytes. J Interferon Cytokine Res 15:739–746
134. Dadmarz R, Sgagias MK, Rosenberg SA, Schwartzentruber DJ (1995) CD4+ T lymphocytes infiltrating human breast cancer recognize autologous tumor in an MHC-class II restricted fashion. Cancer Immunol Immunother 40:1–9
135. Takahashi T, Chapman PB, Yang SY, Hara I, Vijayasaradhi S, Houghton AN (1995) Reactivity of autologous CD4+ T lymphocytes against human melanoma. Evidence for a shared melanoma antigen presented by HLA-DR15. J Immunol 154:772–779
136. Topalian SL, Rivoltini L, Mancini M, Markus NR, Robbins PF, Kawakami Y, Rosenberg SA (1994) Human CD4+ T cells specifically recognize a shared melanoma-associated antigen encoded by the tyrosinase gene. Proc Natl Acad Sci USA 91:9461–9465
137. Topalian SL, Gonzales MI, Parkhurst M, Li YF, Southwood S, Sette A, Rosenberg SA, Robbins PF (1996) Melanoma-specific CD4+ T cells recognize nonmutated HLA-DR-restricted tyrosinase epitopes. J Exp Med 183:1965–1971
138. Kobayashi H, Kokubo T, Sato K, Kimura S, Asano K, Takahashi H, Iizuka H, Miyokawa N, Katagiri M (1998) CD4+ T cells from peripheral blood of a melanoma patient recognize peptides derived from nonmutated tyrosinase. Cancer Res 58:296–301
139. Halder T, Pawelec G, Kirkin AF, Zeuthen J, Meyer HE, Kun L, Kalbacher H (1997) Isolation of novel HLA-DR restricted potential tumor-associated antigens from the melanoma cell line FM3. Cancer Res 57:3238–3244
140. Li K, Adibzadeh M, Halder T, Kalbacher H, Heinzel S, Muller C, Zeuthen J, Pawelec G (1998) Tumour-specific MHC-class-II-restricted responses after in vitro sensitization to synthetic peptides corresponding to gp100 and Annexin II eluted from melanoma cells. Cancer Immunol Immunother 47:32–38
141. Chaux P, Vantomme V, Stroobant V, Thielemans K, Corthals J, Luiten R, Eggermont AM, Boon T, van der Bruggen P (1999) Identification of MAGE-3 Epitopes Presented by HLA-DR Molecules to CD4(+) T Lymphocytes. J Exp Med 189:767–778
142. Manici S, Sturniolo T, Imro MA, Hammer J, Sinigaglia F, Noppen C, Spagnoli G, Mazzi B, Bellone M, Dellabona P, Protti MP (1999) Melanoma cells present a MAGE-3 epitope to CD4(+) cytotoxic T cells in association with histocompatibility leukocyte antigen DR11. J Exp Med 189:871–876
143. Pieper R, Christian RE, Gonzales MI, Nishimura MI, Gupta G, Settlage RE, Shabanowitz J, Rosenberg SA, Hunt DF, Topalian SL (1999) Biochemical identification of a mutated human melanoma antigen recognized by CD4(+) T Cells. J Exp Med 189:757–766
144. Wang R-F, Wang X, Atwood AC, Topalian SL, Rosenberg SA (1999) Cloning genes encoding MHC class II-restricted antigens: mutated CDC27 as a tumor antigen. Science 284: 1351–1354
145. Wang R-F, Wang X, Rosenberg SA (1999) Identification of a novel MHC class II-restricted tumor antigen resulting from a chromosomal rearrangement recognized by CD4+ T cells. J Exp Med 189:1659–1667
146. Nordlund JJ, Kirkwood M, Forget BM, Milton G, Albert DM, Lerner AB (1983) Vitiligo in patients with metastatic melanoma: a good prognostic sign. J Am Acad Dermatol 9: 689–695
147. Nanda NK, Sercarz EE (1995) Induction of anti-self-immunity to cure cancer. Cell 82:13–17
148. Bystryn J-C, Rigel D, Friedman RJ, Kopf A (1987) Prognostic significance of hypopigmentation in malignant melanoma. Arch Dermatol 123:1053–1055
149. Richards JM, Mehta N, Ramming K, Skosey P (1992) Sequential chemoimmunotherapy in the treatment of metastatic melanoma. J Clin Oncol 10:1338–1343
150. Rosenberg SA, White DE (1996) Vitiligo in patients with melanoma: normal tissue antigens can be targeted for cancer immunotherapy. J Immunother 19:81–84
151. Ogg GS, Rod Dunbar P, Romero P, Chen JL, Cerundolo V (1998) High frequency of skin-homing melanocyte-specific cytotoxic T lymphocytes in autoimmune vitiligo. J Exp Med 188:1203–1208
152. Overwijk WW, Tsung A, Irvine KR, Parkhurst MR, Goletz TJ, Tsung K, Carroll MW, Liu C, Moss B, Rosenberg SA, Restifo NP (1998) gp100/pmel 17 is a murine tumor rejection antigen: induction of "Self"-reactive, tumoricidal T cells using high-affinity, altered peptide ligand. J Exp Med 188:277–286

153. Hara I, Takechi Y, Houghton AN (1995) Implicating a role for immune recognition of self in tumor rejection: passive immunization against the *Brown* locus protein. J Exp Med 182:1609–1614
154. Takechi Y, Hara I, Naftzger C, Xu Y, Houghton AN (1996) A melanosomal membrane protein is a cell surface target for melanoma therapy. Clin Cancer Res 2:1837–1842
155. Hirschowitz EA, Leonard S, Song W, Ferris B, Leopold PL, Lewis JJ, Bowne WB, Wang S, Houghton AN, Crystal RG (1998) Adenovirus-mediated expression of melanoma antigen gp75 as immunotherapy for metastatic melanoma. Gene Ther 5:975–983
156. Naftzger C, Takechi Y, Kohda H, Hara I, Vijayasaradhi S, Houghton AN (1996) Immune response to a differentiation antigen induced by altered antigen: a study of tumor rejection and autoimmunity. Proc Natl Acad Sci USA 93:14809–14814
157. Weber LW, Bowne WB, Wolchok JD, Srinivasan R, Qin J, Moroi Y, Clynes R, Song P, Lewis JJ, Houghton AN (1998) Tumor immunity and autoimmunity induced by immunization with homologous DNA. J Clin Invest 102:1258–1264
158. Overwijk WW, Lee DS, Surman DR, Irvine KR, Touloukian CE, Chan CC, Carroll MW, Moss B, Rosenberg SA, Restifo NP (1999) Vaccination with a recombinant vaccinia virus encoding a "self" antigen induces autoimmune vitiligo and tumor cell destruction in mice: requirement for CD4(+) T lymphocytes. Proc Natl Acad Sci USA 96:2982–2987
159. Boon T, Van der Bruggen P (1996) Human tumor antigens recognized by T lymphocytes. J Exp Med 183:725–729
160. Marchand M, van Baren N, Weynants P, Brichard V, Dreno B, Tessier MH, Rankin E, Parmiani G, Arienti F, Humblet Y, Bourlond A, Vanwijck R, Lienard D, Beauduin M, Dietrich PY, Russo V, Kerger J, Masucci G, Jager E, De Greve J, Atzpodien J, Brasseur F, Coulie PG, van der Bruggen P, Boon T (1999) Tumor regressions observed in patients with metastatic melanoma treated with an antigenic peptide encoded by gene MAGE-3 and presented by HLA-A1. Int J Cancer 80:219–230
161. Parkhurst MR, Salgaller M, Southwood S, Robbins P, Sette A, Rosenberg SA, Kawakami Y (1996) Improved induction of melanoma reactive CTL with peptides from the melanoma antigen gp100 modified at HLA-A0201 binding residues. J Immunol 157:2539–2548
162. Banchereau J, Steinman RM (1998) Dendritic cells and the control of immunity. Nature 392:245–252
163. Nestle FO, Alijagic S, Gilliet M, Sun Y, Grabbe S, Dummer R, Burg G, Schadendorf D (1998) Vaccination of melanoma patients with peptide- or tumor lysate-pulsed dendritic cells. Nat Med 4:328–332
164. Zhong L, Granelli-Piperno A, Choi Y, Steinman RM (1999) Recombinant adenovirus is an efficient and non-perturbing genetic vector for human dendritic cells. Eur J Immunol 29:964–972
165. Ockert D, Schmitz M, Hampl M, Rieber EP (1999) Advances in cancer immunotherapy. Immunol Today 20:63–65
166. Timmerman JM, Levy R (1999) Dendritic cell vaccines for cancer immunotherapy. Annu Rev Med 50:507–529
167. Wan Y, Emtage P, Foley R, Carter R, Gauldie J (1999) Murine dendritic cells transduced with an adenoviral vector expressing a defined tumor antigen can overcome anti-adenovirus neutralizing immunity and induce effective tumor regression. Int J Oncol 14:771–7716
168. Kaplan JM, Yu Q, Piraino ST, Pennington SE, Shankara S, Woodworth LA, Roberts BL (1999) Induction of antitumor immunity with dendritic cells transduced with adenovirus vector-encoding endogenous tumor-associated antigens. J Immunol 163:699–707
169. Liu MA (1998) Vaccine developments. Nat Med 4:515–519
170. Ying H, Zaks TZ, Wang RF, Irvine KR, Kammula US, Marincola FM, Leitner WW, Restifo NP (1999) Cancer therapy using a self-replicating RNA vaccine. Nat Med 5:823–827